

INDUCED CROSSING OVER IN DROSOPHILA MALES
AND ITS PROBABLE NATURE

MAURICE WHITTINGHILL

University of Michigan, Ann Arbor, Michigan

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INTRODUCTION

IN AN earlier paper (SHULL and WHITTINGHILL 1934) a preliminary report of the induction of crossing over in *Drosophila melanogaster* males by high temperatures was made. The present account describes a repetition of this experiment on a larger scale, several new attempts to induce crossing over and an interpretation of the results, together with a survey of the extent of sex differences in crossing over.

Sex differences in crossing over

Since the discovery of sex differences in crossing over in *Drosophila* (MORGAN 1912, 1914), there have become available data on the occurrence of such differences in other species, on the cytological accompaniments of crossing over, and on exceptional instances of crossing over in *Drosophila* males.

A brief survey of the relation of sex to crossing over in species where crossing over has been genetically demonstrated will show the degree to which *Drosophila* stands apart in this respect. ELOFF (1932) has written a more detailed treatment of this subject. Aside from several species of *Drosophila*, *melanogaster*, *simulans* and *virilis*, absence of crossing over in one sex is known only for the silkworm, *Bombyx mori* (TANAKA 1915, 1927; OGURA 1932). Five different linkages, in four linkage groups, have been studied in the silkworm without finding any crossing over in females, which are heterozygous for sex, although crossing over occurs in males.

Less extreme differences may be found in three genera in which crossing over, although present in both sexes, is markedly reduced in one of them. In *Apotettix* crossing over is confined almost exclusively to the female (NABOURS 1929). In *Paratettix* the female shows twice as much crossing over as the male (HALDANE 1920, NABOURS 1929). *Gammarus chevreuxi* has the same sex difference, in one instance 25.4 percent crossing over in males compared with 50.4 percent in females (HUXLEY 1928).

Sex differences of smaller magnitude have been debated by investigators of rodents and fowls. For rats and mice CASTLE (1925) has reviewed numerous instances in which differences however slight always show a higher value for crossing over in females. Differences in chiasma frequen-

¹ Contribution from the Department of Zoology, University of Michigan.



cies have been reported to be in the same direction in these two species (BRYDEN 1932, CREW and KOLLER 1932). However, at least one example of lower crossing over in the female rat has been reported between the genes pink and ruby, although the reverse relation was found between pink and albinism, an allele of ruby (FELDMAN 1924). As DETLEFSEN (1925) pointed out, only two of the many linkages measured in rats and mice were sufficiently great to be significant by themselves. That the differences may be intensified by age is suggested by the work of GREEN (1935) showing that age increased the amount of crossing over in females and decreased it in males. Thus no generally acceptable conclusion in regard to these species can be stated at the present time.

In the domestic fowl the situation is similar to that in rats and mice; all the five available reports say that the female sex gave a higher value of crossing over, although the differences were slight and possibly invalidated by the demonstration of age effects. LANDAUER (1931) found the most significant difference of the five between genes showing 0.68 percent crossing over in the female and 0.19 percent in the male in over 2000 gametes each, but even this, as LANDAUER stated, is a slight difference. Computation shows that the above difference is only 2.5 times its standard error. The other differences found (SUTTLE and SIPE 1932, HUTT 1933, WARREN 1935, WARREN and HUTT 1936) were all less than their standard errors. Although a pronounced increase in crossing over with the advancing age of males has been reported for sex-linked genes in males (HALDANE and CREW 1925), in the opposite sex a slight decrease, if anything, has been described (LANDAUER 1933). Thus the evidence for a sex difference in linkage in the fowl, which resembles *Bombyx* in having the ZW type of sex determination, remains inconclusive for the present.

In contrast to the above five or eight genera in which sex differences in linkage strength have been noted, there are five or more other genera which show equal frequencies of exchange. Sex differences in crossing over are believed to be absent in the pigeon (CHRISTIE and WRIEDT 1924), and in the rabbit (CASTLE 1926). Several well studied plants also show no consistent crossover differences in megasporocytes as compared to microsporocytes. Such plants include *Primula sinensis* (DE WINTON and HALDANE 1935), maize, *Antirrhinum*, *Lathyrus odoratus*, *Oryza*, *Pharbitis Nil*, and tomatoes (SANSOME and PHILP 1932). Occasionally, as in *Primula*, higher crossing over in pollen mother cells has been found for certain linkages, but for certain other characters the opposite is true, and with still others equal crossing over has been found.

Two general interpretations of the phenomena described up to this point have been offered by HALDANE (1922) and by HUTT (1933). HALDANE has suggested that wherever crossing over is reduced or absent in one sex the

affected sex is the heterogametic one. Our knowledge of *Drosophila*, *Apotettix*, *Paratettix*, *Gammarus*, which are of XY type, and of *Bombyx*, ZW type, is in agreement with this theory. The other interpretation, advanced by HUTT, is that reduction of crossing over always occurs in the male, regardless of the type of sex determination. This theory is supported by the same list of XY organisms which are also included in HALDANE's generalization, but it receives scant support, if not an outright contradiction, from ZW species. *Bombyx*, with no crossing over in the female, does not find a place in HUTT's picture, although it is included in that of HALDANE. The only support offered for HUTT's contention is the borderline example of the domestic fowl, where the higher crossing over indicated for the female has not been established nearly as well as the reverse case of *Bombyx*. Further studies of crossing over in species in which the female is heterozygous for sex will therefore be of special interest.

Cytological observations

Although the cytological counterpart of the reduction of genetic cross-overs is not completely known in *Drosophila*, because of the difficulty of studying maturation in this genus, some elucidation has been secured. It has been reported by HUETTNER (1930) and by KOLLER and TOWNSON (1933) that meiosis in the male differs from that in the female in that during spermatogenesis synapsis takes place between short lengths of homologous chromosomes at any one time. The pairing begins at the spindle fiber attachments in all chromosomes and moves out to the ends in the autosomes, but is confined to the inert regions of the XY pair. Chiasmata were not observed in the latter study although later described for X and Y in *D. pseudoobscura* (DARLINGTON 1934) and inferred in *D. melanogaster* from the genetic demonstration of crossing over (PHILIP 1935). Chiasmata between autosomes of males have not been described, although rare cases of genetic crossing over have been found there also.

Spontaneous crossing over in Drosophila males

Exceptions in which crossing over, of a spontaneous nature, has occurred in males have been reported about seven times in *Drosophila* literature. The first case described (MULLER 1916) stands apart from the others in that every one of 193 backcross offspring of a single male *D. melanogaster* showed that crossing over between truncate and black had occurred, although in six brother F_1 males segregation was normal. MULLER pointed out that this crossing over must have occurred once very early in the embryonic development of this F_1 male to have affected all of his mature germ cells. A second instance of spontaneous crossing over in this species was the occurrence of a single Star curved speck fly among the offspring of a backcrossed Star speck/purple curved speck male (BRIDGES and MOR-

GAN 1919). In *D. simulans* a single crossover between scarlet and peach appeared among 183 other offspring from the backcross of a single male. The phenomenon did not reappear again in an extensive search for it with the same stocks (STURTEVANT 1929).

In addition to these chance findings other researches directed at the possibility of male crossing over have shown that such exchanges may occur spontaneously at a very low rate. Between the X and Y of *D. melanogaster* double crossing over in the inert region and involving bobbed has been found with a frequency of 1 in 3100 (PHILIP 1935). In the third chromosomes of the same species 1 crossover among 8239 controls was found by PATTERSON and SUCHE (1934). In still another species, *D. virilis*, crossing over at about the same rate as the last, 3 in 38,598, has been reported (KIKKAWA 1933). Therefore crossing over in the male of *Drosophila* is not an impossibility even under natural conditions.

Induced crossing over

Artificial induction of crossing over in males was suggested mainly by the knowledge that in females crossover values could be increased by the use of temperature extremes (PLOUGH 1917, 1921) and X-rays (MAVOR and SVENSON 1924). The production of crossing over in males of *D. melanogaster* has been reported in a substantial percentage of gametes following treatment with X-rays (FRIESEN 1934, PATTERSON and SUCHE 1934) and with high temperature (SHULL and WHITTINGHILL 1934, PLOUGH 1935). In *D. virilis* both agencies have induced crossing over in males to a slight degree (MORIWAKI 1935).

EXPERIMENTS WITH "STUCA" HETEROZYGOTES

Materials and methods

The stocks used in this experiment were one containing the third chromosome recessives thread scarlet curled stripe sooty claret, here referred to as "stuca" and identical with the stock erroneously called "theca" in our previous report (SHULL and WHITTINGHILL 1934), and a wild stock which had been collected locally and which replaced the wild stock formerly used. Five P₁ matings were made using brothers and sisters from each stock: three were "stuca" females by wild males and two were the reciprocal. The five sets of flies were changed at the same time to four successive half-pint culture bottles, in the first of which they remained for two days, in the others for one day each.

Heat treatment consisted in placing the resulting twenty cultures in a breeding cabinet, the temperature of which varied between 33.5° and 35.5°C as determined both by a thermograph and by a mercury thermometer placed between two trays of bottles. Treatment of all bottles

began together and continued for five days for the older cultures and six and one-half days for the younger larvae. Drops of water and of yeast solution were added to the cultures at intervals during heating. When the cultures were removed to normal breeding temperature, 25°C, many or all of the larvae had died. Males hatched from only ten of the thirteen cultures which produced any adults at all.

As many of the adult F_1 males as possible were backcrossed to "stuca." Those which were fertile were transferred from the first vial to a second large culture and then to another vial, which served as storage while the first two cultures were examined for crossover offspring. If none were found, the third cultures were discarded; if crossovers were present, the male producing them was transferred to a new culture bottle every fifth day until after death. One such male was remated to new virgin females.

When crossover offspring were found, they were dealt with in various ways. Homozygous stocks or balanced stocks were made from representative crossovers. Each stock came from a single crossover male in most cases (table I). Where a female was the only crossover of a particular class from one treated male, three separate stocks were established from each of three of her sons to lessen the chance that the region of induced exchange might be crossed out. Moiré, Moiré curled, Moiré curled claret and other derivatives of Mé/"rucuca" and the old wild stocks were used to build up and sometimes to maintain the desired stocks.

The crossovers were distinguished from chromosomal aberrations not only by the viability of homozygotes but by measures of crossing over at and near the region of the induced exchange. A few brief tests were made by backcrossing the daughters of crossovers following suitable matings, but the most extensive tests were made after stocks had been established. Several similar stocks, together with a control of similar genotype though of origin solely through female crossing over, were tested together, making six such groups of tests. P_1 matings were made with one or more flies from the stock to be tested in each vial. Heterozygotes hatched and were bred at a temperature of approximately 25°C, although some of the F_1 underwent their earlier development at temperatures several degrees higher at times. From six to ten F_1 females were backcrossed, two females to four "stuca" males in a half-pint bottle, where they remained three days before being transferred, without etherization, to second and third bottles for two days each. Additional males were introduced when two or more of the originals became useless. All living flies were etherized and examined before being discarded. The resulting offspring were classified until the sixteenth day after starting the culture, or the fifteenth wherever the hatch began as early as the last of the eighth day. The counts tapered off in almost all cases or even fell to zero, so it is believed that all the offspring

TABLE I

Crossovers from "stuca" × Wild Matings 1936.

P ₁ MATINGS	F ₁ MALES BRED	F ₁ MALES WITH CROSSING OVER	CULTURES OF SAID MALE	OFFSPRING OF SAME: PHENOTYPE	TOTALS	DESIGNATION OF DERIVED C. O. STOCK†	VIABILITY OF HOMO- ZYGOTE
K	6	#3	4	wild "stuca"	204 136 <u>1</u> 341	52 ♀	V*
L	8	#6	7	wild "stuca"	540 357	{ 14 38 39 10 ♀ 13 ♀	V V V V L*
				<i>th st cu sr e^s</i>	48		
				<i>ca</i>	<u>1</u>		
					946		
		#7	7	wild "stuca"	394 327	{ 102 23 41 42 43 33 46 48 101 31	L V V V V L L L L ?
				<i>ca</i>	2		
				<i>th st cu</i>	23		
				<i>sr e^s ca</i>	20		
				<i>th st</i>	<u>1</u>		
					767		
		#9	4	wild "stuca"	83 81	4	V
				<i>cu sr e^s ca</i>	<u>2</u>		
					166		
J	5	#20	11	wild "stuca"	508 486	{ 19 50 58 59 17 25 26 27 ♀	V V V V V V V ?
				<i>th st</i>	144		
				<i>cu sr e^s ca</i>	<u>7</u>		
					1145		

* V = viable; L = lethal.

† The derived stocks are from males, unless otherwise designated.

were included in the classification without reducing the representation of any class having slow development. A variation of the above procedure was followed in the first control, C₁, measured independently of any other tests, in that only two successive cultures, of four and three days respectively, were used and in that in half of the backcrosses pair matings were substituted for the two by four plan. From some of the other control tests F₁ males were backcrossed individually to investigate the possibility of lower viability of some classes which were represented only rarely among the offspring of heated males.

Results

The occurrence of induced crossovers and the viability of homozygous stocks derived from representative crossovers is shown in tables 1 and 2. The first section of table 1 reads as follows: From a "stucca" by wild mating, K, six F₁ males were successfully backcrossed, of which one, number 3, showed crossing over. Male number 3 produced, in four successive cultures before he died, 204 wild and 136 "stucca" non-crossovers and one claret crossover. Since this lone crossover (number 52) was a female, homozygous stocks were bred from each of three of her thread scarlet curled claret sons to insure that the affected region near claret would not be lost. Similarly in the next family, L male number 6, stocks were established from the crossovers numbered 14, 38, 39, 10 and 13. Single stocks were established from each crossover listed except those which were females and are so designated in the table. Viable homozygous stocks were obtained for most crossovers, as indicated in the last column.

It will be noted that from L male 7 none of the stocks of stripe sooty claret crossovers could be obtained in a homozygous condition. The lethals were conclusively located in three of the four stocks, 33, 48 and 101, when crossover tests were being made. The method comprised matings back to the lethal stock females of males from both classes of crossovers in regions 5, 4, 3 and 5, 2 and 5. The expected offspring were Moiré, stripe sooty claret and, if the lethal had crossed out of the crossover chromosome, flies showing one or two of these recessives. Absence of this third class would indicate that the lethal-bearing section of the chromosome was still present in spite of the loss of other sections through crossing over. When the results were secured it was found that chromosomes retaining the left hand part of the original chromosome following crossing over in regions 3, 4 and 5, which means between curled and claret, still carried the lethal and that the reciprocal classes were viable in every case as expected. When crossovers for region 2, scarlet to curled, were backcrossed to the lethal stock, results varied from one male to another, showing that crossing over could take place within this marked region and to either side of the lethal.

Thus the lethal was located between st^+ and cu^+ , which was to the left of the region in which induced crossing over had occurred.

Other evidence showed that the lethal was not caused in any way by the heat treatment but was present in the wild stock at the beginning of the experiment. When stocks similar in phenotype to the induced crossover stocks were needed, some of them were made up by the same method of using crossover suppressors as was followed with the derivatives from induced crossovers. Here, as in the experimental series, stripe sooty claret and claret stocks were lethal when homozygous. All curled stripe sooty

TABLE 2
Crossovers from Wild $\times$ "stuca" Mating 1936.

P ₁ MATINGS AND BOTTLE NO.	F ₁ MALES BRED	F ₁ MALES WITH CROSSING OVER	CULTURES FROM SAID MALE	OFFSPRING OF SAME: PHENOTYPE	TOTALS	DESIGNATION OF DERIVED C. O. STOCK†	VIABILITY OF HOMO- ZYGOTE
H1	I	#4	3	wild "stuca" <i>th st cu sr e^s</i>	111 98 2	{ 3 36 6 ♀ 37	V V ? L
				<i>ca</i>	2		
					213		
H4	II	#31	3	wild "stuca" <i>th st</i>	211 165 1	I	?
				<i>cu sr e^s ca</i>	0		
					377		

† The derived stocks are from males, unless otherwise designated.

claret stocks, some of which might have carried a lethal located as above, were free from it. No representatives of the complementary classes of any of these three had any lethals, with the possible exception of one curled stripe sooty claret and two thread scarlet stocks not satisfactorily bred.

While the evidence shows that the lethal carried by L male 7 was located between the loci of st and cu , a different lethal, located to the right of cu , must have been carried by L male 6. For two *th st cu ca* sons of the female crossover 13 still carried lethals in spite of the loss of the region to the left of cu . In the absence of information about the precise location of this lethal, it is not possible to decide whether the induction of crossing over had anything to do with its origin.

It is remarkable that most of the positive results came from P₁ matings of "stuca" females by wild males (table 1), and a very few from the

reciprocal matings (table 2). The same situation prevailed in the earlier experiment in 1934. This statement holds whether we compare the num-

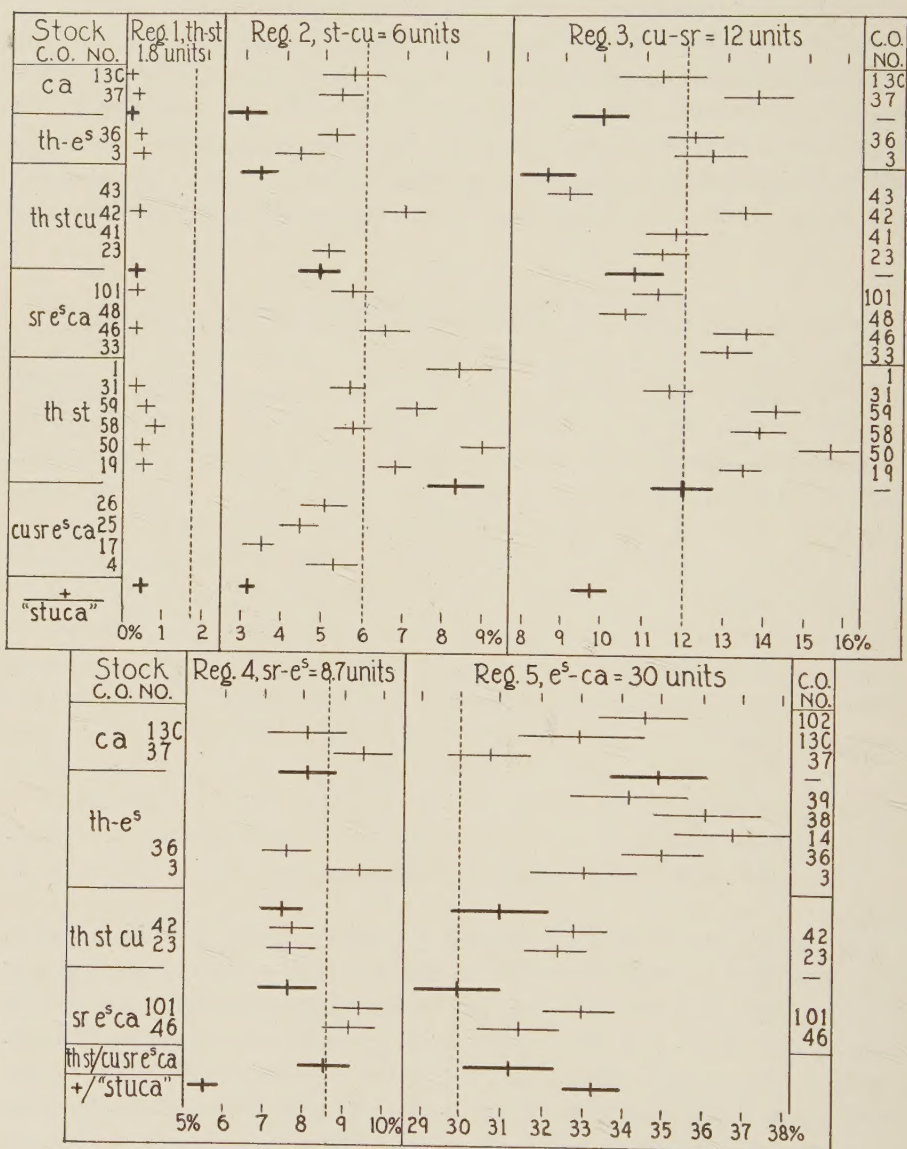


FIGURE 1.—Distribution of crossing over in derived stocks in various regions. Vertical lines, measured crossing over; horizontal lines, standard errors above and below measured crossing over; heavy lines, control stocks; light lines, derived stocks; dotted lines, map values.

bers of crossovers, 249 to 5 this year and 46 to 34 earlier, or the numbers of males in which crossing over to any extent was induced, 5/19 vs. 2/12 recently and 10/28 vs. 3/38 formerly. In other words, males from "stuca"

mothers and wild fathers were three times as susceptible to the induction of crossing over by heat as were males from the reciprocal matings.

Measurements of crossing over in the region of the induced exchange, and often in other adjacent regions, were made in order to discover whether the percentages might be abnormal because of gross chromosomal changes such as deficiencies, terminal or lateral duplications, or translocations. The results (fig. 1) do not seem to indicate any such reductions in crossing over as would be expected if chromosomal abnormalities were acting. Although considerable variation is evident, it should be noted that the five controls show almost as great a range of variability in each region as do the various derived stocks, and that crossing over in the region of the previously induced crossing over was not noticeably more different from its proper control than was the crossing over in neighboring regions. The reason for the variability found may have been differences in physiological age (BRIDGES 1929) of females hatching at the same time from cultures of different ages or of females whose early larval development was passed at temperatures higher than the optimum, regardless of the fact that they awaited hatching at a temperature of 25°C.

EXPERIMENTS WITH OTHER STOCKS

Induction of crossing over in heterozygotes from another third chromosome multiple recessive stock was attempted on as large a scale as the previous experiment. Matings were made between the wild stock used above and Moiré/"rucuca" stock, which contained recessives like those in "stuca" with the addition of roughoid and hairy, marking the left arm of the third chromosome. One fly of crossover appearance, roughoid hairy thread scarlet, hatched but was sterile. Another had rough eyes but did not pass the character on to later generations. A third also had rough eyes together with swollen wing veins, the two being due to one dominant gene later determined by its position in the chromosome to be the mutation Delta. Temperatures of 9°C were tried in addition to and instead of the high temperatures but without positive results.

A few crossovers between second chromosome genes were induced in males from a mating of Star females by Curly/dumpy purple plexus males. Three Star dumpy purple flies came from a single male, following treatment for four hours at 38°C. These were due to exchanges between the Star and dumpy loci, although exact tests to prove the normality of subsequent crossing over in this region were not performed. The lack of plexus was not due to double crossing over but, as BEDICHEK (1936) has shown, to suppression by the gene Star, although in our results homozygous plexus was not suppressed in about five percent of the flies also bearing Star. Other crossovers were found in this experiment, but because some of them bore

the dominant Curly, introduced by the females from the poorly viable and therefore balanced dumpy purple plexus stock used for the backcross, the remaining crossovers, non-Star and non-Curly, might have arisen through crossing over in parents of either sex. Only the three Star dumpy purple (suppressed plexus) flies were definitely due to exchanges in the male.

One other attempt to induce crossing over was made, this time on the X and Y. A stock of eosin Bar was made up to provide males to be mated, after heating, to another stock in which had been incorporated yellow, white and miniature. In an extensive search no crossovers were found, although they have recently been demonstrated (PHILIP 1935) near the locus of bobbed, farther to the right. An eye mutation occurred here in an untreated chromosome at the locus of lozenge.

DISCUSSION

The results produced in induction experiments differ from those of normal crossing over in females in three conspicuous respects. Crossing over in any one male seems to occur in only one region. Secondly, complementary crossover classes may be very unequally represented. Finally, the spindle fiber region is more susceptible to the induction of crossing over than are other regions.

Limitation of crossing over to one region in one male points either to the predetermination or, preferably, to the actual occurrence of crossing over *before* the maturation divisions of the male. Predetermination of crossing over would call for a permanent weakening of an early gonial chromosome whose many daughter chromosomes at meiosis would recombine at the same locus. However, equal complementary classes would be expected from such behavior, whereas they were often found to be unequal. Nor can we rely upon localization of synapsis, for KOLLER and TOWNSON (1933) have observed cytologically that adjacent regions synapse successively in *D. obscura*.

It is simpler to assume that crossing over and segregation may occur only once, in an early spermatogonial cell, resulting in many similar crossovers in the mature sperm. For that matter, one might assume that the spermatids keep dividing for half a dozen generations to produce the same result, but the supporting evidence is stronger for the other alternative. Pairing of homologous chromosomes occurs in somatic as well as in meiotic divisions (METZ 1916, METZ and NONIDEZ 1921, KAUFMANN 1934), and the four-strand stage has been seen by KAUFMANN in somatic cells. Somatic segregation of sex-linked characters has been found in females by PATTERSON (1930) and in both sexes by STERN (1935). That a similar

crossing over and segregation may occur in the germ line is shown by the occurrence of 100 percent crossing over in one male (MULLER 1916).

Early gonial crossing over has been further indicated by other researches. ZELENY (1921) and STURTEVANT (1928) have both suggested it as a cause of multiple occurrences of mutations in the Bar series, which were shown to be dependent upon crossing over. The rare disjunction of attached-X chromosomes, known to depend upon crossing over with a Y, has also occurred more than once in a culture from a single female, obviously from a prematuration exchange (NEUHAUS 1936). Others who have reported the grouping of detachments are L. V. MORGAN (1925), MULLER and DIPPEL (1926), STURTEVANT (1931) and RHOADES (1931). The rarely occurring nondisjunction of bobbed deficiency was found twice in two males out of seven showing any at all (GERSHENSON 1935). Correlations between the finding of several similar rare single crossovers from normal heterozygous females have been shown at great length by SEREBROVSKY (1927). Finally, FRIESEN (1934) with his X-ray induced crossovers adds substantial evidence for the grouping of crossovers. Seven of the eight males which showed crossing over revealed it for only one region. In our own experiments strict localization was observed in 17 of the 19 males which produced any crossovers at all.

A second difference between induced and normal crossing over was the presence of unequal complementary classes in some of our results, which also will be explained, later, on the same hypothesis of spermatogonial crossing over. The complementary classes from 4 males were very unequal, from 6 others they were essentially equal, and from the remaining 9 there were too few crossovers to be significant. Of the inequalities two showed 10 percent and 12.5 percent of thread scarlet flies to 1 percent or none of the reciprocal class. A third contrasted 5 percent of the thread scarlet curled stripe sooty class with 0.1 percent of claret, while a fourth reversed these proportions to zero and 1 percent. FRIESEN did not encounter the phenomenon, but PATTERSON'S gross results showed totals of 27 roughoid hairy thread scarlet flies to 6 of the complementary curled stripe sooty claret class.

Because the differences were in the same direction in PATTERSON and SUCHE'S investigation and in our own, the question was asked whether viability differences could account for any of them. Examination of various complementary classes in different control backcrosses of F_1 females revealed none of suggestive nature, so males heterozygous for various complementary classes and brothers of the control females were also backcrossed. Two *th st/cu sr e^s ca* males, backcrossed individually, gave a total of 212:213 of the expected classes. Several *th st cu/sr e^s ca* males pro-

duced the expected classes to the extent of 129 and 120. Two *th st cu sr e^s/ca* males, individually mated, gave a ratio of 219:197. Transmission through the sperm was therefore normal, and the cause of the inequalities had to be sought elsewhere.

On the assumption of the premeiotic occurrence of crossing over these inequalities might arise from differential division rates of the two gonial cells receiving the segregated crossover chromosomes. This interpretation is supported by the fact that flies of the deficient classes appeared at the beginning or at the end of the family, in the five places in which they appeared at all, and never in the middle, although the more abundant class of crossovers hatched at a rather regular frequency throughout all or nearly all cultures. Thus two of the peculiarities of induced crossovers from males can be explained best on the assumption that crossing over may be induced in gonial cells at from one to six or more cell generations before the first meiotic divisions.

The third difference between induced crossing over in males and normal crossing over in females is that the spindle fiber vicinity is more responsive to alterations of frequency than are other parts of the chromosome. In FRIESEN'S, PATTERSON and SUCHE'S and our own experiments the numbers of crossover flies for spindle fiber regions of even short lengths comprised half of the total or more. Restating the data on the basis of only one crossing over being the source of any number of flies of two complementary crossover classes does not materially alter the picture. FRIESEN'S results reveal that spindle fiber regions could satisfactorily account for eight of the ten sources of crossover flies. On the same explanation our own crossovers can all be the result of ten occurrences of somatic crossing over in as many different males in the short spindle fiber region, seven occurrences in the curled-sooty region, over three times as long, and of seven exchanges in the distal sooty-claret region, five times as long as the first region.

This well known condition where the spindle fiber region is the part of the chromosome most susceptible to changes in crossover frequency brought about by heat, cold, X-rays, age and starvation makes one wonder whether these induced changes in females may not be more like the induced crossovers in males than like normal crossing over in females.

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SUMMARY

1. Crossing over has been induced in *Drosophila melanogaster* males by temperatures of 35°C applied during the larval period. This parallels the action of X-rays on the male and of heat on the female.

2. Crossing over was found almost exclusively in males heterozygous for the third chromosome recessives *th st cu sr e^s ca*, here called "stuca," although a few apparent crossovers appeared from a second chromosome heterozygote, *S/dp pr px*. None were found from heterozygous "rucuca" males. This difference may be explained by postulating an enhancer in the "stuca" stock.

3. Males from "stuca" by wild matings produced three times as much crossing over as males from the reciprocal matings, as measured either by the numbers of crossover offspring or the proportion of males affected to males bred. This indicates that the enhancer had a maternal or sex-linked effect.

4. Crossing over was observed to be concentrated in the spindle fiber region, although found also in the middle and terminal regions.

5. The induced-crossover chromosomes showed no abnormal effects upon phenotype, upon viability of the homozygotes nor upon subsequent crossing over. On this basis the terms crossovers and crossing over are regarded as applicable.

6. In individual males crossing over occurred in only one region in 17 of the 19 which produced crossovers.

7. Complementary crossover classes were usually both present, but were very unequal in most families of males producing more than five percent of crossovers.

8. The last two points can best be explained on the hypothesis, suggested by several other investigators, that the more numerous induced crossovers arose from a single crossing over which occurred in a spermatogonial cell several cell generations before meiosis. The scattered crossovers, one or two from a male, might have originated then or at maturation equally well.

BIBLIOGRAPHY

- BEDICHEK, SARAH, 1936 Suppression of the recessive plexus by the dominant Star in the II chromosome of *Drosophila melanogaster*. D. I. S. 5: 24 (by author's permission).
- BRIDGES, C. B., 1929 Variation in crossing over in relation to age of female in *Drosophila melanogaster*. Pub. Carnegie Instn. 399: 63-89.
- BRIDGES, C. B., and MORGAN, T. H. 1919 The second chromosome group of mutant characters. Pub. Carnegie Instn. 278: 123-304.
- BRYDEN, WM., 1932 Cytogenetic studies on the rat. Somatic chromosome complex, meiosis and chiasma frequency. J. Genet. 26: 395-415.

- CASTLE, W. E., 1925 A sex difference in linkage in rats and mice. *Genetics* **10**: 580-582.
 1926 Studies in color inheritance and linkage in rabbits. *Pub. Carnegie Instn.* **337**: 1-47.
- CHRISTIE, W., and WRIEDT, C., 1924 Die Vererbung von Zeichnungen, Farben und anderen Charakteren bei Tauben. *Z. i. A. V.* **32**: 233-298.
- CREW, F. A. E., and KOLLER, P. C., 1932 The sex incidence of chiasma frequency and genetical crossing over in the mouse. *J. Genet.* **26**: 359-383.
- DARLINGTON, C. D., 1934 Anomalous chromosome pairing in the male *Drosophila pseudoobscura*. *Genetics* **19**: 95-118.
- DETLEFSEN, J. A., 1925 The linkage of dark-eye and color in mice. *Genetics* **10**: 17-32.
- ELOFF, G., 1932 A theoretical and experimental study on the changes in the crossing over value, their causes and meaning. *Genetica* **14**: 1-116.
- FELDMAN, H., 1924 Linkage of albino allelomorphs in rats and mice. *Genetics* **9**: 487-492.
- FRIESEN, H., 1934 Künstliche Auslösung von Crossing Over bei *Drosophila*-männchen. *Biol. Zbl.* **54**: 65-75.
- GERSHENSON, S., 1935 Possible role of the genetically inert region of the X chromosome in equational divisions. *D. I. S.* **4**: 64 (by author's permission).
- GREEN, C. V., 1935 Apparent changes with age in crossing over between color and size genes in mice. *J. Genet.* **30**: 101-106.
- GUYENOT, E., and NAVILLE, A. 1929 Les chromosomes et la réduction chromatique chez *Drosophila melanogaster*. *La Cellule* **39**: 27-82.
- HALDANE, J. B. S., 1920 Note on a case of linkage in *Paratettix*. *J. Genet.* **10**: 47-51.
 1922 Sex ratio and unisexual sterility in hybrid animals. *J. Genet.* **12**: 101-109.
- HALDANE, J. B. S. and CREW, F. A. E., 1925 Change of linkage in poultry with age. *Nature* **115**: 641.
- HUETTNER, A. F., 1930 The spermatogenesis of *Drosophila melanogaster*. *Zeit. Zellf. mikr. Anat.* **11**: 615-637.
- HUTT, F. B., 1933 Genetics of the fowl. II. A four-gene autosomal linkage group. *Genetics* **18**: 82-94.
- HUXLEY, J. S., 1928 Sexual difference of linkage in *Gammarus chevreuxi*. *J. Genet.* **20**: 145-156.
- KAUFMANN, B. P., 1934 Somatic mitosis of *Drosophila melanogaster*. *J. Morph.* **56**: 125-156.
- KIKKAWA, H., 1933 Crossing over in the male of *Drosophila virilis*. *Proc. Imp. Acad. Tokyo* **9**: 535-536.
- KOLLER, P. C., and TOWNSON, THELMA, 1933 Spermatogenesis in *Drosophila obscura* Fallen. I. The cytological basis of suppression of crossing over. *Proc. Roy. Soc. Edinb.* **53**: 130-146.
- LANDAUER, W., 1931 The linkage relationship of the autosomal genes for creeper and rose comb in the fowl. *Anat. Rec.* **51** (Supplement): 123.
 1933 Creeper and single comb linkage in fowl. *Nature* **132**: 606.
- MAVOR, J. A., and SVENSON, H. K., 1924 An effect of X-rays on the linkage of Mendelian characters in the second chromosome of *Drosophila melanogaster*. *Genetics* **9**: 70-89.
- METZ, C. W., 1916 Chromosome studies in the Diptera II. The paired association of chromosomes in the Diptera, and its significance. *J. Exp. Zool.* **21**: 213-279.
- METZ, C. W. and NONDEZ, JOSÉ F., 1921 Spermatogenesis in the fly, *Asilus sericeus* Say. *J. Exp. Zool.* **32**: 165-185.
- MORGAN, L. V., 1925 Polyploidy in *Drosophila melanogaster* with two attached-X chromosomes. *Genetics* **10**: 148-178.
- MORGAN, T. H., 1912 Complete linkage in the second chromosome of the male of *Drosophila*. *Sci.* **36**: 719-720.
 1914 No crossing over in the male of *Drosophila* of genes in the second and third pairs of chromosomes. *Biol. Bull.* **26**: 195-204.
- MORIWAKI, D., 1935 Crossing over in the male of *Drosophila virilis* induced by heat and X-rays. *Proc. Imp. Acad. Tokyo* **11**: 242-243.
- MULLER, H. J., 1916 The mechanism of crossing over. *Amer. Nat.* **50**: 284-305.
- MULLER, H. J. and DIPPEL, A. L., 1926 Chromosome breakage by X-rays and the production of eggs from genetically male tissue in *Drosophila*. *Brit. J. Exp. Biol.* **3**: 85-122.

- NABOURS, R. K., 1929 The genetics of the Tettigidae (Grouse Locusts). *Bibliogr. Genet.* **5**: 27-104.
- NEUHAUS, M. J., 1936 Crossing over between the X and Y chromosomes in the female of *Drosophila melanogaster*. *Z.i.A.V.* **71**: 265-275.
- OGURA, SABURO, 1932 Erblchkeitsstudien am Seidenspinner *Bombyx mori* L. III. *Z.i.A.V.* **61**: 315-408.
- PATTERSON, J. T., 1930 Somatic segregation produced by X-rays in *Drosophila melanogaster*. *Proc. Nat. Acad. Sci.* **16**: 109-111.
- PATTERSON, J. T. and SUCHE, META L., 1934 Crossing over induced by X-rays in *Drosophila* males. *Genetics* **19**: 223-236.
- PHILIP, URSULA, 1935 Crossing over between X and Y chromosomes in *Drosophila melanogaster*. *J. Genet.* **31**: 341-352.
- PLOUGH, H. H., 1917 The effect of temperature on crossing over in *Drosophila*. *J. Exp. Zool.* **24**: 147-209.
- 1921 Further studies on the effect of temperature on crossing over. *J. Exp. Zool.* **32**: 178-202.
- 1935 Crossing over in male *Drosophila melanogaster* following heat. *D. I. S.* **3**: 50 (by author's permission).
- RHOADES, M. A., 1931 The frequencies of homozygosis of factors in attached-X females of *Drosophila melanogaster*. *Genetics* **16**: 375-385.
- SANSOME, F. W., and PHILP, J., 1932 Recent Advances in Plant Genetics. 414 pp. London: J. and A. Churchill.
- SEREBROVSKY, A. S., 1927 The influence of the purple gene on the crossing over between black and cinnabar in *Drosophila melanogaster*. *J. Genet.* **18**: 137-175.
- SHULL, A. FRANKLIN, and WHITTINGHILL, M., 1934 Crossovers in male *Drosophila melanogaster* induced by heat. *Science* **80**: 103-104.
- STERN, CURT, 1935 Further studies on somatic crossing over and segregation. *Amer. Nat.* **69**: 81-82.
- STURTEVANT, A. H., 1928 A further study of the so-called mutation at the Bar locus of *Drosophila*. *Genetics* **13**: 401-409.
- 1929 The genetics of *Drosophila simulans*. *Pub. Carnegie Instn.* **399**: 1-62.
- 1931 Two new attached-X lines of *Drosophila melanogaster* and further data on the behavior of heterozygous attached-X's. *Pub. Carnegie Instn.* **421**: 61-81.
- SUTTLE, A. D., and SIPE, G. R., 1932 Linkage of genes for crest and frizzle in domestic fowl. *J. Hered.* **23**: 135-142.
- TANAKA, Y., 1915 Occurrence of different systems of gametic reduplication in male and female hybrids. *Z.i.A.V.* **14**: 12-30.
- 1927 Some remarks on symbols, multiple allelomorphism, crossing over and linkage groups in the silkworm. *J. Dept. Agric. Kyushu Imp. Univ.* **1**: 275-279.
- WARREN, D. C., 1935 A new linkage group in the fowl (*Gallus domesticus*). *Amer. Nat.* **69**: 82.
- WARREN, D. C. and HUTT, F. B., 1936 Linkage relations of crest, dominant white and frizzling in the fowl. *Amer. Nat.* **70**: 379-394.
- DE WINTON, DOROTHY, and HALDANE, J. B. S., 1935 The genetics of *Primula sinensis*. III. Linkage in the diploid. *J. Genet.* **31**: 67-100.
- ZELENY, C., 1921 The direction and frequency of mutation in the Bar-eye series of multiple allelomorphs of *Drosophila*. *J. Exp. Zool.* **34**: 203-233.

